BioSEA Instruments in BRC

Angelo Benedetto, Ph.D.
Research Scientist
26 January, 2012
BioSEA Open House
Flow Cytometry

BD FACSCanto II Flow Cytometer

• 2-lasers, 6-colors
• FACSDiva collection software
• FlowJo analysis software
Beckman Coulter Avanti J-E Centrifuge

- 4 x 1L @ 9000 rpm
- 6 x 500mL @ 10000 rpm
- 6 x 250mL @ 10000 rpm
- 8 x 50mL @ 20000 rpm
- Temperature Range: –10 to 40°C
Centrifugation

Beckman Coulter Avanti J-HC Centrifuge

- 4 x 2.25L @ 5000 rpm
- Temperature Range: −10 to 40°C
Beckman Coulter Optima L-90K Ultracentrifuge

- 8 x 13.5mL @ 65000 rpm
- 6 x 5mL @ 55000 rpm
- 6 x 94mL @ 45000 rpm
- 6 x 38.5mL @ 32000 rpm
- 8 x 39mL @ 70000 rpm
- Temperature Range: 0 to 40°C
Beckman Coulter Proteomelab XL-A Analytical Ultracentrifuge

- 1 or 3 cells at once
- Spectral Range: 190 – 800 nm
  Usable Range: 230 – 650 nm
- Absorbance only
- 45000 or 60000 rpm
  (dependent on cell)
Particle Sizing / Zeta Potential

Malvern Zen 3600 Zetasizer

- Dispersed particles
- Molecules in solution
- Size, molecular wt., zeta potential
Colloidal Dynamics Model 25 Zeta Potential Analyzer

- Particles > 50 μm
- Fibers
- Flat Surfaces
- Streaming zeta potential
- Currently coming back online
Optical Microscopy

Zeiss Axiovert 200M Inverted Fluorescence TIRF Microscope

• Simultaneous TIRF and epi-fluorescence imaging
• HAL 100 Module
• HBO 100 Module
• TIRF Laser Module
Optical Microscopy

Zeiss Axioimager Z2 Microscope

• Color and monochrome cameras
• Fluorescence and halogen sources
• Automated filter cube turret
• Multiple objectives available
Optical Imaging

FujiFilm LAS 4000 Imager

- Chemiluminescence
- Bioluminescence
- Fluorescence
- Cooled 3.2 megapixel CCD
Animal Imaging

Maestro Small Animal Imaging System

• Whole animal (mouse) imaging
• Fluorescence detection of nanomaterials in living animals
• Use subject to regulations and approval
Spectrofluorometry

SPEX FluoroLog-3 Spectrofluorometer-NIR
Spectrofluorometry

SPEX FluoroLog-3 Spectrofluorometer-NIR/UV-vis
Spectrophotometry

Nanodrop 2000

• Sample volume as low as 0.5 μL
• Spectral Range: 190 – 840 nm
• Currently restricted to nucleic acids
• Not currently on SEA website
QSense E4 Quartz Crystal Microbalance

- Automated real-time surface analysis
- Mass and viscoelastic properties of changing molecular layers
- Various substrates available
BioRAD CFX96 PCR Real Time System

- Sample volume as low as 10 μL
- Thermal cycling in <30 minutes
- Temperature Range: 0 to 100°C
- Not currently on website
BioRAD C1000 Thermal Cycler

• Adjacent to PCR
• Not currently on website
Scintillation Counting

Beckman Coulter LS6500 Scintillation Counter
Sample Preparation and Storage

Labconco Freezone 4.5 Plus Freeze Dryer

• Not currently on website
Sample Preparation and Storage

New Brunswick Scientific Excella E25 Incubator Shaker

- Flasks up to 6L
- Temperature Range: 4 – 60°C
- Orbital diameter of 1”
- Speed: 50 – 400 rpm
Sample Preparation and Storage

VWR -86°C Freezer
BioNMR Facility

Dr. Quinn Kleerekoper
NMR Manager
Keck Hall Rm B08
qkk1@rice.edu
NMR Spectrometer
Keck Hall

Howard Keck Hall

Visitor parking is indicated by yellow shaded areas. The Rice shuttle visits all parking facilities every five minutes, and also stops directly in front of Keck Hall.
NMR Instrumentation

500 MHz

600 MHz
800 MHz
Probes

Optimal for Biomolecular Applications

Penta probes are factory tuned to allow decoupling of up to four different nuclei, including $^2$H lock, and are highly versatile for biomolecular applications. The inner coil is optimized for $^1$H sensitivity with excellent RF homogeneity, $^{31}$P, and $^2$H decoupling. The outer coil is optimized for short $^{13}$C and $^{15}$N 90° pulse widths. The Penta probe is ideal for both protein and nucleic acid research applications.
Cold Probes

4X SENSITIVITY over conventional probes
Advantages of Cold Probes

Optimized for Biomolecule Applications

The 5 mm Triple Resonance Cold Probes provide extreme sensitivity for biomolecule applications, such as proteins and RNA/DNA, or natural products. Probes are configured standard as $^1$H{$^{13}$C/$^{15}$N} and optimized for $^1$H observations with the capability for simultaneous or single irradiation of $^{13}$C and $^{15}$N frequencies. These probes are equipped with a high-performance, actively-shielded Z-axis gradient.

600 MHz : Cooled C13 Preamplifier – improved C13 sensitivity
Ideal for Biological Samples

- 500 MHz
- 600 MHz
- 800 MHz

Signal/Noise:
- 800
- 4100
- 6000
How much sample do I need?

- Concentration range from 2 µM to 1mM
- Volume range 300 to 600 µL
- MW limits upwards of 60 kD for structures (84kD protein solved)
- Typically, due to low natural abundance of $^{15}$N (0.37%) and $^{13}$C (1.1%) isotopes, recombinant proteins are prepared in minimal medium containing $^{13}$C carbon source and $^{15}$NH$_4$Cl.
What can NMR do for me?

1D $^1$H-NMR spectrum

2D $^{15}$N-HSQC spectrum
NMR spectra
NMR spectra

2D $^{13}$C-HSQC spectrum
What simple questions can we ask?

- Is my protein folded?
  screening site directed mutants
- Is my protein aggregated?
- How stable is my protein?
- Is my protein dynamic?
- Does my protein form a complex?
  Ligand binding – drugs e.g.

Two excellent review articles on biological NMR for non-spectroscopist are the following:
Kwan et al FEBS Journal 278 (2011) 687-703
Bieri et al FEBS Journal 278 (2011) 704-715
Screening site-directed mutants

Wild-type

Mut A

Mut B

Mut C

Mut D

Mut E

$^1$H chemical shift (ppm)
Deletion of Asp 470 Causes Global Structural Changes in T3-Cterm

Kleerekoper et al JBC 277 (2002) 10581
Structural Basis of Bone Disease
Use of $^{13}$C HSQC to Monitor Drug Binding to Cardiac Troponin C
Where do Calcium Sensitizing Compounds Bind on Cardiac Troponin C

Met Methyl Groups in cTnC

Calcium Sensitizing Compounds

- Trifluoperazine
- Bepridil
Metabolically Label Cardiac Troponin C with \([^{13}\text{C}]-\text{Methyl Methionine}\)

**Met Methyl Groups in cTnC**

**HSQC of \([^{13}\text{C}]-\text{methyl Met cTnC}\)**
Met Methyl Chemical Shifts are Affected by Drug Binding
cTnC Binds Three Molecules of Bepridil or TFP
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Uses of NMR Spectroscopy in the Biosciences

Most all activities require some amount of atom-specific resonance assignments. For proteins ~30 kDa, this process can be automated up to a point, provides a wealth of information, and is a mechanism to enhance grant proposals and recruit collaborators for more advanced studies, but requires on-site expertise (for non-experts) and a few hundred instrument hours (A1 below).

A. Biomolecular Structure & Dynamics
   1. Resonance Assignments
      4-8 3D spectra and >10 2D spectra (200-600 instrument hours)
   2. Structure Data (NOEs, J-couplings, RDCs, CSA)
      6-12 3D spectra (300-800 instrument hours)
   3. Dynamics Data (T1, T2, heteronuclear nOe, relaxation-dispersion)
      30+ 2D & 3D spectra (200-1000 instrument hours)

B. Macromolecular Survey
   1. ligand binding, folding, complex formation, pilot studies
      2D hetero- and homo-nuclear (2+ instrument hours per sample)
   2. High-throughput screening, structure-activity relationship
      2D hetero-nuclear (2+ instrument hours per sample)

C. Small Molecule (Natural Product) Analysis
   1D and some 2D (15’+ instrument time per sample)

D. Metabolomics
   Quantitative carbon/nitrogen/phosphorus flux
   1D and 2D (15’+ instrument time per sample)

Types of Macromolecular Service
-Is my protein NMR amenable in this buffer?
   1-2 hr of 2D HSQC (15-30 minute staff time)

-Does this mutant affect structure/dynamics?
   1-2 hr of 2D HSQC (15-30 minute staff time)

-What is the secondary structure of my <25kDa protein?
   4-6 3D experiments, 6-72 hr each
   (15-30 minute setup time each; 1-2 hr processing time each; 1-2? hr using autoassign)

-Does molecule A interact with molecule B?
   1-2 hr of 2D HSQC (15-30 minute staff time)

-Can you collect 1D spectra of my “metabolomic” samples?
   5-60’ per sample (5 minute setup; 0-30 analysis)
Transmission Electron Microscope

Dr. Wenhua Guo
TEM Manager
How it works
JEOL 1230 High Contrast TEM

Specs:
• 60-120kV; Tungsten Filament; CCD camera

Capabilities:
• Imaging, diffraction, tomography
JEOL 2011 Cryo TEM/JEOL 2010 TEM
Specs:
• 100-200KV, LaB6 Filament, CCD Camera
Capabilities:
• Imaging, diffraction, Cryo.

CDF image B = 111

TEM BF Cryo Image of SWNTs in water solution
JEOL 2100F EFTEM

Specs:
• 80-200kV, Field Emission Gun, STEM, GIF/EELS, EDS

Capabilities:
• HR-Imaging, STEM imaging, Diffraction, EELS/EDS mapping
Vivarium Collaborations

Kelly Campbell, RLATG
Manager, Animal Resources Facility
713-348-3502
Kellyc@rice.edu
BRC Core Mass Spec Facility, Instrumentation and Applications

Christopher L. Pennington, Ph.D.

01/26/2012

BioSEA Open House
MS Core Lab Overview

• **Instrumentation details: 4 MS systems in total**
  – Ionization techniques: ESI, APCI, MALDI, APPI, DART
  – Two systems w/ accurate mass & MS/MS capabilities (IT-ToF, Orbitrap)
  – Two systems w/ LC-MS capabilities (IT-ToF, MicroToF)
  – Two walk up systems (MicroToF, Autoflex)
    • Routine analysis (MW determination, did I make my product?)
    • Contact: Christopher Pennington ([clp5@rice.edu](mailto:clp5@rice.edu)) or Gloria Funderburg ([gloriaf@rice.edu](mailto:gloriaf@rice.edu))
  – Two stand alone computer for data analysis

• **Types of molecules analyzed**
  – Small Molecules (polar)
  – Proteins & Peptides
  – Polymers (polar, nonpolar)
  – Nucleotides
  – Glycans
  – Petro Chem
  – Fullerenes

• Facility staff contribute to projects on a fee for service and collaborative basis.
MALDI MS Protein & Peptide Analysis

Angio II

Peptide CAL Mix II

BSA Tryptic Digest

Intact Protein BSA 66 KDa
Fyn SH3 modification through dirhodium catalyzed C – H insertion of carbene

Crystal structure of Fyn SH3 showing two tryptophan side chains, the presumed modification site(s).

Courtesy of Farrukh Vohidov & Prof. Zachary Ball, Rice University
MALDI MS Protein Modification Reaction Monitoring

Fyn SH3 domain sequence

GSAMTGVLFTVL.ValYDYEARTED
DLSFHKGKQFQILNSSEGDWWE
ARSLTGETGYIPSNYVAPVDSI

Mw = 7438.13

t = 10 min

t = 1 h

t = 3 h

t = 5 h

+1 mod

+2 mod

+1 mod

+1 mod

+2 mod

Courtesy of Farrukh Vohidov & Prof. Zachary Ball, Rice University
Modification of Acyl Carrier Protein Lct26

• Lct26 (MW = 10405) is acyl carrier protein (ACP) from a polyketide pathway that is associated with fatty acid biosynthesis.

• Activation of Lct26 occurs via posttranslational phosphopantetheinylation
  – phosphopantetheinyl transferase (PPTase)
  – Sfp transferring phosphopantetheinyl group from CoA to the active site serine of the apo-ACP
  – Expected MW shift from apo to holo is 340 Da

• In vitro experiment Lct26:
  – Apo Lct26: expressed it in E coli, isolated, and purified by HPLC.
  – Holo Lct26: reaction was carried out in by incubating Apo Lct26, CoA, and Sfp to generate Holo Lct26, which was isolated by HPLC.

In collaboration with Dr. Jane Coughlin and Prof. Ronald Parry, Rice University
**Calculated MW:** 10406  
_Proposed MW:_ 10405  
_Error(%): 0.01%  
_Error(ppm): 100 ppm

_In collaboration with Dr. Jane Coughlin and Prof. Ronald Parry, Rice University_
**Calculated MW:** 10746
Proposed MW: 10745
Error(%): 0.01%
Error(ppm): 100 ppm

**Mass Difference Calculated**
HOLO 26 – APO 26 = 10746 – 10406 = 340 Da
Consistent with proposed modification

*In collaboration with Dr. Jane Coughlin and Prof. Ronald Parry, Rice University*
POG-\(^{13}\)C Peptide
Determining Site of \(^{13}\)C Label

C\(_{119}\)\(^{13}\)CH\(_{173}\)N\(_{31}\)O\(_{40}\)
Exact Mass: 2689.2490

- Goal: confirm the site of the \(^{13}\)C labeled glycine residue for the synthetic peptide POGPOGPOGPOGPOGPOGPOGPOGPOGPOG-NH\(_2\), where O is hydroxyproline.

- LC-MS/MS was used to determine if \(^{13}\)C label was present on at Gly12, Gly15, or Gly18 residue.

- MS/MS data was exported to mMass and matched against theoretical fragmentation patterns for differently labeled \(^{13}\)C POG peptides (Gly12, Gly15, or Gly18)

In collaboration with Abhishek Anan & Prof. Jeffrey Hartgerik, Rice University
POG-\(^{13}\)C Peptide MS Spectrum

In collaboration with Abhishek Anan & Prof. Jeffrey Hartgerik, Rice University

C\(_{119}\)\(^{13}\)CH\(_{173}\)N\(_{31}\)O\(_{40}\)
Exact Mass: 2689.2490

Obs Mass: 2689.2453
\(\Delta\)ppm = 1.39
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In collaboration with Abhishek Anan & Prof. Jeffrey Hartgerik, Rice University
Peak Matching Using mMasa
Semi Automated Data Analysis

In collaboration with Abhishek Anan & Prof. Jeffrey Hartgerik, Rice University

Comparing Fragmentation Pattern Matches

The MS/MS data supports the conclusion that the 13C labeled Gly residue is Gly15.

- **Fragment Ions Matched**
  - $^{13}$C Gly-12
  - $^{13}$C Gly-15
  - $^{13}$C Gly-18
- **MS/MS results indicate** $^{13}$C MOD is on Gly-15

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In collaboration with Abhishek Anan & Prof. Jeffrey Hartgerik, Rice University
Small Molecule Characterization & Structure Elucidation

LC-MS/MS Characterization of Methylone Analogues

- Methyleneoxy-2-aminoindane (MDAI)
  Chemical Formula: C₁₅H₁₁NO₂
  Exact Mass: 177.0790
- Methoxetamine (MXE)
  Chemical Formula: C₁₅H₂₁NO₂
  Exact Mass: 247.1572
- Methyleneoxypropylamine (MDPV)
  Chemical Formula: C₁₅H₂₁NO₃
  Exact Mass: 275.1521
- Methiopropamine (MPA)
  Chemical Formula: C₈H₁₃NS
  Exact Mass: 155.0769
- Dimethylamylamine (DMAA)
  Chemical Formula: C₇H₁₇N
  Exact Mass: 115.1361
### MS Spectrum & Characterization of MDPV

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<td>C16H21NO3</td>
<td>[M]+</td>
<td>276.1583</td>
<td>119.596</td>
<td>119.596</td>
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<td>119.596</td>
<td>119.596</td>
<td>1.00</td>
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#### 277.1617

**Δppm = 3.98**

Methylenedioxyprovalerone (MDPV)
Chemical Formula: C16H21NO3
Exact Mass: 275.1521
2797 matches to the chemical formula $\text{C}_{16}\text{H}_{21}\text{NO}_{3}$
MS/MS

m/z 276.1583

Methylenedioxyxypovalerone (MDPV)
Chemical Formula: C_{18}H_{21}NO_3
Exact Mass: 275.1521

<table>
<thead>
<tr>
<th>m/z</th>
<th>Abs. Inten.</th>
<th>Rel. Inten.</th>
</tr>
</thead>
<tbody>
<tr>
<td>126.1262</td>
<td>1102433</td>
<td>26.79</td>
</tr>
<tr>
<td>135.0423</td>
<td>1495220</td>
<td>36.34</td>
</tr>
<tr>
<td>147.0778</td>
<td>275039</td>
<td>6.68</td>
</tr>
<tr>
<td>175.0733</td>
<td>4115010</td>
<td>100</td>
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<tr>
<td>205.0825</td>
<td>3418519</td>
<td>83.07</td>
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Precursor 276.1583
MS Stage 2
m/z with 25 ppm mass accuracy at Positive Mode ALL

Metabolite(s) with containing 4 fragments:

<table>
<thead>
<tr>
<th>Frag. m/z</th>
<th>Δppm</th>
<th>Intensity</th>
<th>CE</th>
<th>Predicted Ion Type</th>
<th>Predicted Fragment Structure</th>
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<tbody>
<tr>
<td>126.1280</td>
<td>14</td>
<td>100.0</td>
<td>10, 20, 40</td>
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<tr>
<td>135.0440</td>
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<td>80.3</td>
<td>10, 20, 40</td>
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<tr>
<td>205.0600</td>
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<td>51.2</td>
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<tr>
<td>147.0780</td>
<td>1</td>
<td>6.2</td>
<td>20, 40</td>
<td>No Structure Information</td>
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</tbody>
</table>

http://metlin.scripps.edu/fragment_search_multi.php

Thermo Mass Frontier
Rationalizing Fragment Ions
Rationalizing Fragmentation for MS/MS of $m/z$ 276.1583

<table>
<thead>
<tr>
<th>m/z</th>
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<th>Rel. Inten.</th>
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<td>100</td>
</tr>
<tr>
<td>205.0825</td>
<td>3418519</td>
<td>83.07</td>
</tr>
</tbody>
</table>

Precursor: $m/z$ 276.1583
MS Stage: 2
Data Analysis Software Programs & Databases

• **Vendor Software**
  – Bruker
    • FlexAnalysis (Autoflex),
    • TargetAnalysis (MicroToF)
  – Thermo Fisher
    • Xcalibur (LTQ-Orbitrap)
    • Xcalibur FT Tools (Advanced high resolution data processing)
    • Mass Frontier (MS/MS data interpretation, fragment ion prediction)
  – Shimadzu Scientific
    • Lab Solutions (IT-ToF)
    • Formula Predictor (Accurate mass & isotope pattern used to propose chemical formulas)
    • MetID solutions (Metabolite profiling)
    • Profiling Solutions (LC-MS chromatogram comparisons)
  – Matrix Science: Mascot Distiller v. 2.3.2.0
    • Mascot Search Tool Box (Protein data base searching of MS data)
    • De Novo Search Tool Box (de Novo peptide sequencing)

• **Open Access Resources, Software & Databases**
  – METLIN [http://metlin.scripps.edu/metabo_search_alt2.php](http://metlin.scripps.edu/metabo_search_alt2.php)
  – HMDB [http://www.hmdb.ca/search/spectra?type=ms_search](http://www.hmdb.ca/search/spectra?type=ms_search)
  – Delta Mass (Protein PTMs) [http://www.abrf.org/index.cfm/dm.home](http://www.abrf.org/index.cfm/dm.home)
Acknowledgements

• SEA Board
• Rice Faculty & Administration
  – Prof. Vicki Colvin (Vice Provost for Research)
  – Prof. Cindy Farach-Carson (Vice Provost Translational Bioscience)
  – Prof. Doug Natelson (Chair, SEA Board)
  – Dr. Wade Adams (Associate Dean, Engineering)
  – Prof. Zachary Ball (Chemistry)
  – Prof. George Bennett (Biochemistry & Cell Biology)
  – Prof. Paul Engel (Chemistry)
  – Carlos Garcia, Jr. (Office of Research)
  – Prof. Ramon Gonzalez (Chemical & Biomolecular Engineering)
  – Prof. Jeffrey Hartgerink (Chemistry)
  – Prof. James McNew (Biochemistry & Cell Biology)
  – Prof. Ronald Parry (Chemistry)
  – Prof. Ka-Yiu San (Bioengineering)
  – Prof. Rafael Verduzco (Chemical & Biomolecular Engineering)

• SEA Research Scientists & Staff
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  – Gloria Funderburg
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  – Dr. Quinn Kleerekoper
  – Dr. Gang Liang

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  – Dr. Erica Bakota (USDA)
  – Dr. Jane Coughlin (Chemistry)
  – Brian Grindel (Biochemistry & Cell Biology)
  – Stacy Prukop (Chemical Engineering)
  – Dr. Leepika Tuli (Bioengineering)
  – Farrukh Vohidov (Chemistry)
<table>
<thead>
<tr>
<th>Name</th>
<th>e-mail address</th>
<th>Major Facilities</th>
</tr>
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<tbody>
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</tr>
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<td><a href="mailto:bochen@rice.edu">bochen@rice.edu</a></td>
<td>XPS, BET</td>
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<td>TEM</td>
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<td>SEM, AFM, Raman, TGA, FTIR</td>
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<td>Mass Spectrometry</td>
</tr>
<tr>
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<td><a href="mailto:kelly.m.campbell@rice.edu">kelly.m.campbell@rice.edu</a></td>
<td>Vivarium</td>
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</tbody>
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